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Epidemiological Study of the G Serotype Distribution of Group A Rotaviruses in Kenya From 1991 to 1994

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An epidemiological study on the G serotype distribution of group A rotaviruses (GARV) isolated in Kenya was carried out in one urban hospital in Nairobi and in two rural hospitals in Nanyuki and Kitui to clarify the prevalent G serotypes before future introduction of the ready licensed rotavirus vaccine in Kenya. A total of 1,431 stool specimens were collected from children, who were mainly outpatients, aged from 0 to 6 years old with acute gastroenteritis from August 1991 to July 1994. Samples positive for GARV by conventional ELISA were then analyzed by subgrouping and serotyping ELISA and by PAGE. To ascertain the G serotypes of viruses in samples that were unable to be typed by serotyping ELISA, polymerase chain reaction was also attempted. The prevalence of GARV was 28.4% in the urban hospital, 22.5% in Nanyuki, and 13.7% in Kitui. Among rotavirus-positive samples, subgroup II rotaviruses were detected in 63.1%, and subgroup I rotaviruses were 25.9%. Serotype G4 was most prevalent, accounting for 41.6% followed by 23.3% of serotype G1, 17.0% of serotype G2, and serotype G3 was rarely isolated. Seven strains of serotype G8/P1B rotavirus was detected for the first time in Kenya by RT-PCR. Eleven specimens with an unusual composition of subgroup, serotype, and electropherotype were atypical GARV in which the P-serotype was P1A, P1B, or P2. Although uncommon GARV serotype G8/P1B and atypical GARV were detected, the four major GARV serotypes, G1 through G4, should be targeted at this moment for vaccination to control this diarrheal disease in Kenya. Continuous monitoring of the G- and P-serotype distribution of GARV should provide important information about the impact of rotavirus vaccination in Kenya. ***J. Med. Virol. 58:296–303, 1999.*** © 1999 Wiley-Liss, Inc.

KEY WORDS: group A rotavirus; acute gastroenteritis; Kenya; G serotype; serotype 8; RT-PCR

INTRODUCTION

Group A rotavirus (GARV) gastroenteritis is a worldwide disease affecting primarily infants and young children [Kapikian and Chanock, 1996]. Due to the significant morbidity and mortality caused by GARV gastroenteritis, especially in developing countries, there is great need for studies on these viruses with the goal of developing effective methods for prevention and control of this disease [Kapikian, 1994; Estes, 1996].

GARVs are classified into serotypes defined by the reactivity of viruses in plaque reduction or fluorescent foci reduction neutralization assays, which can measure the reactivity of antibody with the two outer capsid-neutralizing antigens, VP4 (P serotype) and VP7 (G serotype) [Estes, 1996]. Many studies [Offit and Clark, 1985; Chiba et al., 1986; Scaller et al., 1992] have clearly demonstrated the importance of G serotype-specific immunity for protection against rotavirus illness. At least 10 G serotypes (serotypes 1, 2, 3, 4, 5, 6, 8, 9, 10, 12) have been identified in humans, but G serotypes 1, 2, 3, and 4 have been reported to be the most prevalent ones throughout the world [Estes, 1996; Kapikian and Chanock, 1996]. Based on the above epidemiological data and insufficient induction of cross-protection among G serotypes by monovalent rotavirus vaccines, quadrivalent RRV-human rotavirus reassortant vaccines have been developed and field-tested [Kapikian, 1994; Joensuu et al., 1997; Perez-Schael et al., 1997; Desselberger, 1998].

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Several studies on the epidemiology of GARV diarrhea in Kenya have been carried out [Mutanda, 1980; Makino et al., 1983; Miyazaki et al., 1984; Urasawa et al., 1987; Gatheru et al., 1992], while only a single, small-scale epidemiological study on G serotyping of GARVs has been reported [Urasawa et al., 1987]. G serotype distribution should be continuously monitored in each country because uncommon G serotypes, for which the quadrivalent RRV-human rotavirus reassortant vaccines may be ineffective, can be prevalent in some regions, especially in developing countries [Urasawa et al., 1990; Ramachandran et al., 1996]. This article reports the distribution of GARV G serotypes isolated in Kenya before the introduction of rotavirus vaccines in this country.

MATERIALS AND METHODS

Hospitals

The urban area was served by the Infectious Disease Hospital (IDH) in Nairobi, a branch of Kenyatta National Hospital which is the major referral hospital in Kenya. Two district hospitals in Nanyuki and Kitui, which are located 250 and 160 km northeast and east of Nairobi, respectively, served rural areas. This study was conducted for 2 years, from August 1991 to July 1993, in two rural hospitals and for 3 years, from August 1991 to July 1994, in the urban hospital, IDH.

Stool Specimens

A total of 1,431 stool specimens were collected from patients, who were mainly outpatients, aged 0–6 years attending the hospitals with complaints of diarrhea between August 1991 to July 1994. Because our surveillance started in August 1991, the period from August to the next July was defined as one season in this study. The sample size of stool specimens was between 40 and 60 every month except in April and July during the first 2 years and then decreased to about 25 during the third season. Only one stool specimen was collected from each patient. The specimens were stored at –20°C in the rural hospitals until they were transported to the Virus Research Centre (VRC) of the Kenya Medical Research Institute in Nairobi where they were stored at –80°C until processed. Specimens from IDH in Nairobi were transported daily to the VRC.

Laboratory Analysis

Approximately 10% stool suspensions were prepared in 10 mM phosphate-buffered saline (pH 7.4), then extracted with trichlorofluoroethane (Diflon, Daikin Kogyo Co., Japan) and clarified by centrifugation at 7,000*g* for 20 minutes. The aqueous phase was examined for GARV antigen by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Dakopatts, Denmark) following the instruction manual [Grauballe et al., 1981]. Subgroup and G serotype of the GARV-positive stool specimens were analyzed by the ELISA method, using monoclonal antibodies specific for G serotypes 1, 2, 3, and 4, and subgroups I and II, which were produced in Sapporo, Japan [Taniguchi et al., 1984; Urasawa et al., 1988]. All stool samples were examined also by the ELISA for enteric adenoviruses using monoclonal antibodies to either type 40 or type 41 adenovirus [Takagi et al., 1991], and by the antigen ELISA for Sapporo human calicivirus (recently designated "Sapporo virus" as type species of Genus Sapporo-like viruses in Family *Caliciviridae*) [Nakata et al., 1988].

Genomic RNAs were extracted from 0.5 ml of the GARV-positive stool suspensions by using a phenol-chloroform method at first, and later by a RNaid kit (Funakosi Co., Japan); extracted nucleic acid was precipitated with 100% ethanol at –20°C. The pattern of 11 dsRNA segments of GARV was analyzed by polyacrylamide gel electrophoresis (PAGE) [Nakata et al., 1986]. The gels were stained with silver nitrate by using a commercial kit (Ag-STAIN "DAIICHI," Daiichi Pure Chemicals, Japan).

Reverse transcription-polymerase chain reaction (RT-PCR) for G serotyping (1, 2, 3, 4, 8, 9) was carried out in 109 specimens whose G serotype had not been determined by the serotyping ELISA. RT-PCR for P serotyping was carried out in 27 specimens that were composed of 11 GARV strains of unusual constellations, 7 G8 strains, and 9 GARV strains of usual constellations. RT-PCR was performed as previously described [Gouvea et al., 1990b; Taniguchi et al., 1992; Wu et al., 1994] with minimal modifications. Genomic dsRNA for RT-PCR was extracted from 0.1 ml of stool samples by using the RNaid kit.

RESULTS

Prevalence of Gastroenteritis Viruses in Kenya

Figure 1 shows the prevalence of gastroenteritis viruses in stool samples obtained from children younger than 6 years old who had acute gastroenteritis from August 1991 to July 1994. GARVs were the predominant virus detected every year followed by HuCVs and enteric adenoviruses (type 40 and type 41).

Monthly Prevalence of GARVs in the Three Hospitals

The monthly prevalence of GARV in each hospital is shown in Figure 2. GARVs were detected throughout the year in at least one of the three hospitals with a peak occurring in August on average for the three hospitals. Detection rates of GARV during the 3-year study periods demonstrated that there may be two peaks of epidemic periods: from August to September and from February to March, and two peaks of lower detection periods, from May to June and from November to January. Only two seasons, a dry season and a rainy season, are observed in Kenya. The dry season in Kenya consists of 6 months from January to March and from August to October, while the rainy season consists of the 6 months from April to July and November to December. As indicated in Table I, there were statistically significant more GARV infections during the dry seasons in IDH and Nanyuki. By contrast, in Kitui, a district much drier than the other two areas and with

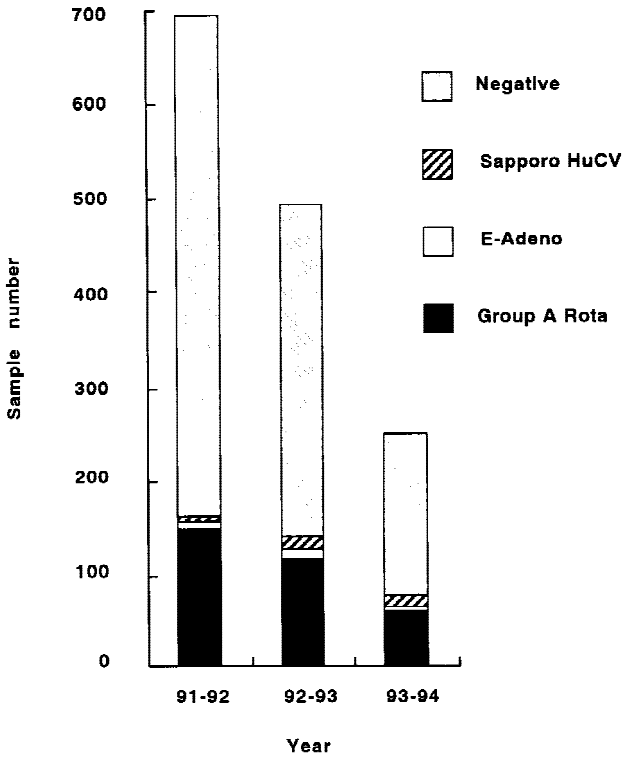

Fig. 1. The prevalence of gastroenteritis viruses in stool samples obtained from children younger than 6 years old with acute gastroenteritis from March 1991 to July 1994 in Kenya.

little rainfall throughout the year, GARV positive rates were low regardless of the climate.

Distribution of Subgroups, G Serotypes and Electropherotypes of GARV Strains Detected in the Three Hospitals

Out of 1,431 stool specimens collected during the 3-year period, 317 (22.2%) were positive for GARV by ELISA. The highest prevalence of GARV was observed at 28.4% in the urban hospital in Nairobi followed by Nanyuki at 22.5% and finally at 13.7% in Kitui (Table II). Subgroup II GARVs were more prevalent compared to subgroup I in any of the hospitals during two or three seasons. About 80% of the GARV strains were electropherotyped by PAGE analysis and a long pattern was about three times more prevalent than a short pattern. Only a few strains were detected that showed a discrepancy among subgroup specificity, G serotype and electropherotype (e.g., where subgroup I specificity correlated with serotype G1 and a long electropherotype). Usually, in humans, subgroup I specificity correlates with a short electropherotype and serotype G2, while subgroup II specificity correlates with a long electropherotype and G serotypes 1, 3, and 4.

Figure 3a shows the yearly changing pattern of the prevalence of G serotypes 1, 2, 3, 4, and 8 in the three hospitals during three seasons, and Figure 3b, c, d shows the pattern by area. G serotypes 1, 2, and 4 strains were detected in the three hospitals, but G serotypes 3 and 8 were detected only in Nairobi (Fig. 3b, c, d). Serotype G4 was the predominant type detected (42.3%), followed by serotype G1 (23.7%), serotype G2 (16.7%), serotype G8 (2.2%), and serotype G3 (0.6%) in the three hospitals.

When the data was analyzed by area, the distribution of each G serotype was generally similar to those found in the three hospitals while serotype G1 strains were more prevalent throughout the three seasons in Nairobi (Figure 3b). In Nanyuki, G serotypes 4 and 2 were detected in about equal numbers followed by a few serotype G1 viruses in the first season; serotype G4 became almost 100% in the second season (Fig. 3c). In Kitui, serotype G4 was much more prevalent, followed by G serotypes 1 and 2 in the first season, and these three G serotypes were detected almost equally in the second season (Fig. 3d).

When the prevalence of each G serotype was examined by year, serotype G4 was most predominant followed by G serotype 1 in the first and second seasons; serotype G1 became more prevalent in the third season with a decrease in the relative frequency of serotype G4 (Fig. 3a). Serotype G2 was steadily detected between 13.8% and 19.3% during the three seasons. Serotype G3 was rarely isolated throughout the study period. An increased tendency to detect serotype G8 was observed in the third year of the study.

Eleven specimens showed an unusual composition of subgroup, G serotype, and electropherotype and indicating these might be atypical GARVs. Three of them had subgroup II, serotype G2, and long electropherotype specificity; one subgroup I, serotype G1, and long electropherotype specificity; three subgroup I, serotype G1, and short electropherotype specificity; three subgroup I, serotype G1, and unknown electropherotype; one subgroup I, unknown serotype, and long electropherotype. The P types of these viruses were determined as shown in Table III. We are now trying to propagate these viruses in tissue culture system for further analysis.

Of 109 specimens (68.8%), 75 whose G serotype was not determined by serotyping ELISA were successfully serotyped by RT-PCR and these results were already included in Table II. Serotype G8 rotavirus strains, uncommonly found in humans in the world, were discovered for the first time in Kenya by RT-PCR. All of these strains have subgroup I, serotype P1B and a short electropherotype specificity (Table III).

DISCUSSION

GARV-positive samples from Kenya were examined at first by serotyping ELISA and only 65.6% (208/317) of them were successfully serotyped. Introduction of RT-PCR improved the sensitivity of G serotyping (85.8%:272/317). Moreover, the RT-PCR method had the advantage over the current available ELISA of being able to determine G serotype more broadly by adding primer sets for new targeting serotypes like G8 and G9 [Taniguchi et al., 1992]. Gastroenteritis was also

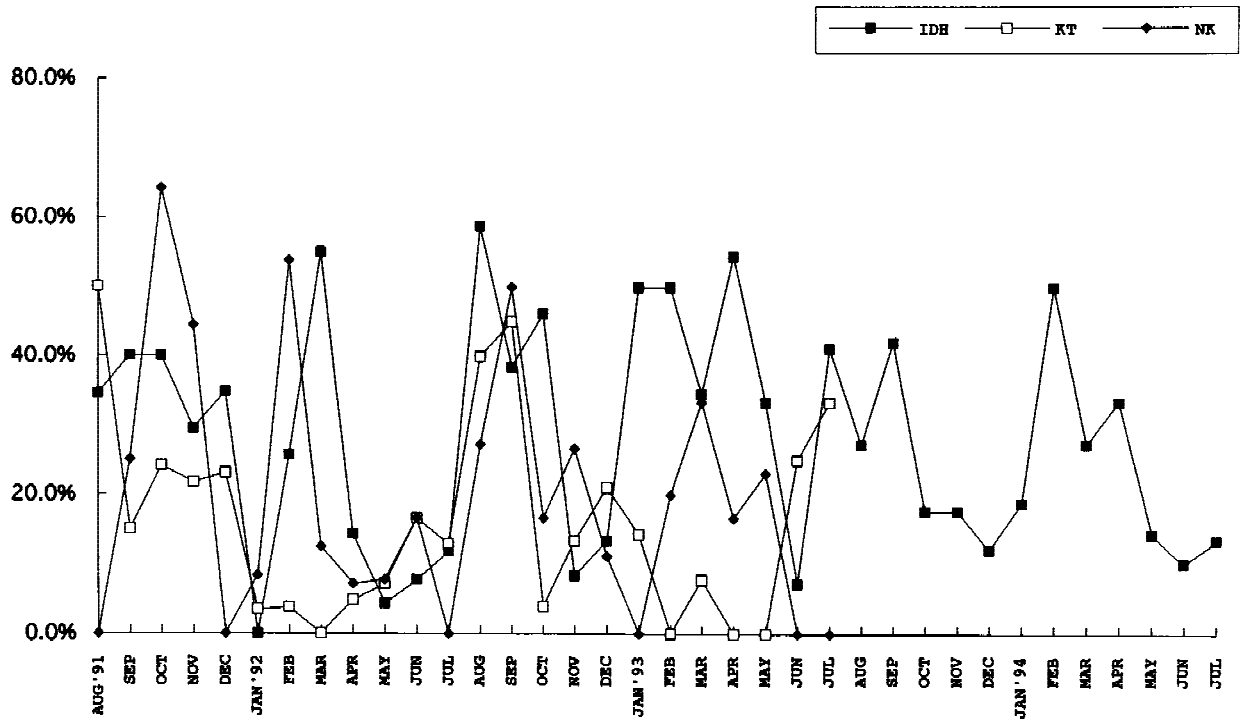

Fig. 2. The monthly prevalence of rotaviruses collected from patients with diarrhea in the Infectious Disease Hospital (IDH), Kitui (KT), and Nanyuki (NK) hospitals from August 1991 to July 1994. ■ IDH; □ Kitui hospital; ◆ Nanyuki hospital.

TABLE I. Comparison of Prevalence of Group A Rotavirus by Two Different Climatological Seasons, Dry and Rainy Seasons, in the Three Hospitals in Kenya

Hospital	Nanyuki (%)	Kitui (%)	Nairobi (IDH) (%)	Total (%)
Dry season[a]	30.2*	13.2	36.8**	45.8***
Rainy season[b]	14.0*	14.1	20.5**	17.1***
Total	22.5	13.7	28.4	22.2

[a]Dry season: Jan, Feb, Mar, Aug, Sep, and Oct.
[b]Rainy season: Apr, May, June, July, Nov, and Dec.
*, **, ***$P < .01$ (chi-square test).

caused by Sapporo calicivirus in this population as discussed elsewhere [Nakata et al., 1998].

The four major G serotypes were the main rotaviruses detected during the three seasons of this study with serotype G4 being predominant followed by G serotypes 1 and 2, and lastly serotype G3 in the three areas. Serotype G3 rotavirus was not detected in the two rural areas and was detected rarely in Nairobi. The findings of a predominance of serotype G4 and an absence of serotype G3 in the two rural areas may be transitional and may change in the future because G serotype predominance depends on the locale and the time as has been observed in both the developing [Urasawa et al., 1987; Ahmed et al., 1989] and the developed countries [Urasawa et al., 1989; Gouvea et al., 1990a; Matson et al., 1990; Bishop et al., 1991; Noel et al., 1991].

The detection of the uncommon serotype G8 rotavirus in Kenya was not unexpected because G serotypes 5, 6, 8, 9, 10, 12, which are common in animals, have been isolated often in humans in other developing countries so far [Matsuno et al., 1985; Clark et al., 1987; Urasawa et al., 1990; Beards et al., 1992; Gerna et al., 1992; Gouvea et al., 1994]. Characterization of such G serotype strains that are uncommon in humans but usually found in animals is interesting because interspecies transmission of rotaviruses may result in antigenic and genetic changes of human rotaviruses [Urasawa et al., 1992] as seen for influenza viruses.

In addition, the prevalence of serotype G8 rotavirus in Nairobi showed an increasing tendency in the last two seasons, and this could become a more prevalent serotype in the future. Serotype G12 rotaviruses were predominantly detected in the feces of children with diarrhea in Metro Manila in Philippines, between December 1987 and February 1988 [Urasawa et al., 1990]. In India, serotype G9 rotaviruses were the most prevalent between April and December 1993 [Ramachandran et al., 1996]. The appearance of uncommon G serotype strains should be monitored carefully to assess their increase or decline. Therefore, continuous monitoring of G serotypes is necessary in all countries, especially in developing countries, to obtain important information for the choice and the impact of rotavirus vaccines in the future.

Electropherotyping of the GARV strains by PAGE is important not only for the analysis of genetic changes but also for the distinction of atypical GARVs from the commonly observed typical GARVs [Aijaz et al., 1996]. The combined analysis of virus strains by PAGE, subgrouping, and serotyping ELISA can detect atypical

TABLE II. Prevalence of Group A Rotavirus and Distribution of Subgroup, G Serotype, and Electropherotype in Diarrheal Stool Samples Collected From Children in Nanyuki and Kitui District Hospitals From August 1991 to July 1993 and Infectious Disease Hospital in Nairobi From August 1991 to July 1994

Hospital	Nanyuki	Kitui	Nairobi (IDH)	Total
No. of stool specimens collected	285	490	656	1,431
No. of rotavirus positive specimens (%)	64 (22.5)	67 (13.7)	186 (28.4)	317 (22.2)
Subgroup (%)				
I	16 (25.0)	13 (19.4)	52 (28.0)	82 (25.9)
II	43 (67.1)	48 (71.6)	112 (60.2)	200 (63.1)
ND	5 (7.8)	6 (9.0)	22 (11.8)	35 (11.0)
Serotype (%)[a]				
1	2 (3.1)	13 (19.4)	60 (32.3)	75 (23.7)
2	14 (21.9)	10 (14.9)	29 (15.6)	53 (16.7)
3	0 (0.0)	0 (0.0)	2 (1.1)	2 (0.6)
4	43 (67.2)	34 (50.7)	57 (30.6)	134 (42.3)
8	0 (0.0)	0 (0.0)	7 (3.8)	7 (2.2)
ND	5 (7.8)	10 (14.9)	31 (16.7)	46 (14.5)
Electropherotype (%)[b]				
Short	13 (20.3)	12 (17.9)	40 (21.5)	65 (20.5)
Long	40 (62.5)	45 (67.2)	102 (54.8)	187 (59.0)
ND	11 (17.2)	11 (16.4)	44 (23.7)	66 (20.8)

ND, not determined.
[a]Of 109 rotavirus-positive stool samples untypable by ELISA, 75 (69%) were successfully typed by PCR. Twelve samples untypable by ELISA could not be tested by PCR due to the scarcity of the specimen.
[b]One sample from Kitui showed a mixed electropherotype of Long and Short.

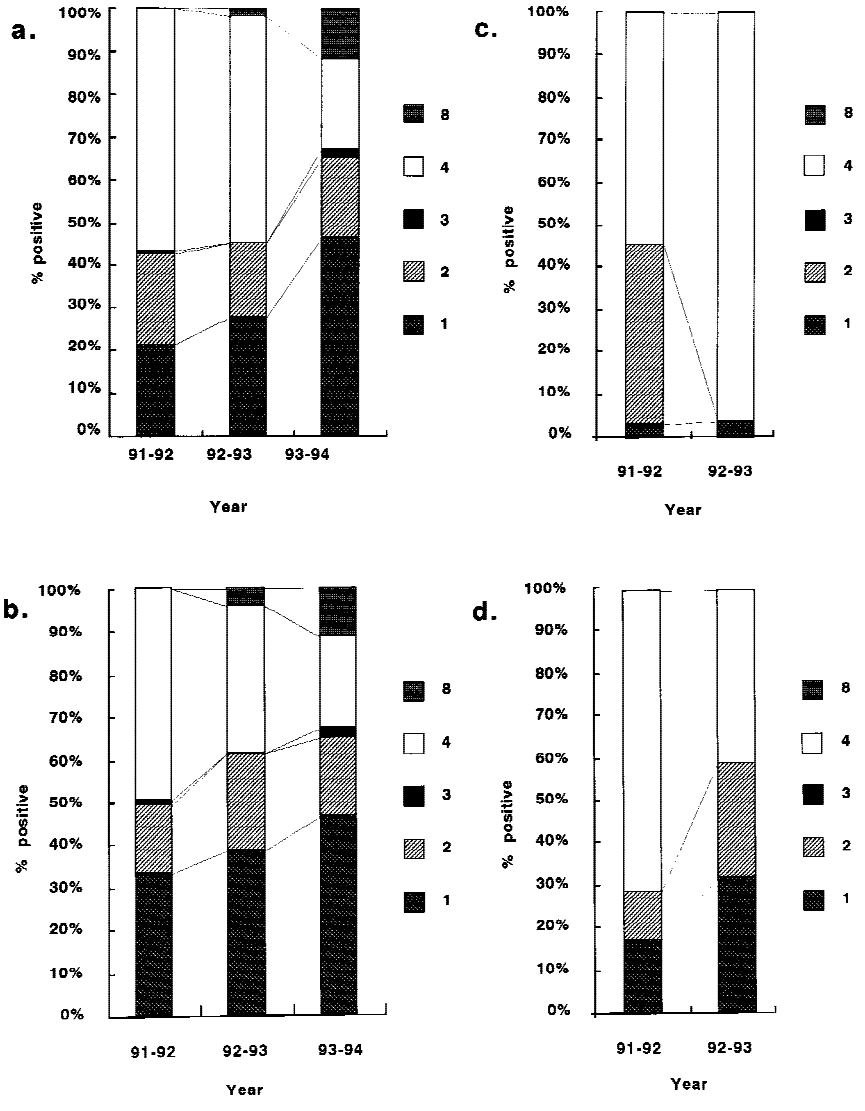

Fig. 3. The yearly changing pattern of the prevalence of G serotypes 1, 2, 3, 4, and 8 in Kenya **(a)** and in Nairobi **(b)** from August 1991 to July 1994, and in Nanyuki **(c)** and in Kitui **(d)** from August 1991 to July 1993.

TABLE III. Correlation of Subgroup, G Serotype, P Serotype, and Electropherotype of Eleven GARV Strains of Unusual Constellations, Seven G8 Strains, and Nine GARV Strains of Usual Constellations

Sample code number	Subgroup	G serotype	P serotype	Electropherotype
GARV strains of unusual constellations				
594	I	1	P1B	?
938	I	1	P1A	Long
1143	I	1	P1B	?
1325	I	1	P1B	Short
1327	II	2	P1A	Long
1383	I	1	P2	?
1416	II	2	P2	Long
1419	II	2	P2	Long
1462	I	?	P2	Long
1466	I	1	P1B	Short
1475	I	1	P1B	Short
G8 GARV strains				
1290	?	8	P1B	Short
1312	I	8	P1B	Short
1345	I	8	P1B	Short
1363	I	8	P1B	Short
1463	I	8	P1B	?
1507	I	8	P1B	Short
1508	I	8	P1B	Short
GARV strains of usual constellations				
1394	II	1	P1A	Long
1467	II	1	P1A	Long
1582	II	1	P1A	Long
1388	I	2	P1B	Short
1405	I	2	P1B	Short
1442	I	2	P1B	Short
1371	II	4	P1A	Long
1482	II	4	P1A	Long
1498	II	4	P1A	Long

GARVs [Estes, 1996]. Eleven GARVs with atypical relationships among these characteristics were detected during our study periods and we are now trying to propagate these viruses in tissue culture using MA-104 cell lines. Two atypical GARVs have already been isolated and characterized in Kenya; one strain (subgroup II, serotype G4) possessed an additional RNA band on PAGE, and the other strain which had an antigenic specificity of subgroup II and serotype G1 showed a "short" RNA pattern [Gatheru et al., 1993].

P serotyping of the serotype G8 strains found rarely in humans and the GARV strains of unusual constellations is interesting because reassortment among human rotaviruses or between human and animal rotaviruses can result in the evolution of new strains with unusual combinations of G and P serotypes [Estes, 1996]. A subset of such strains may adapt to infect humans and become the predominant strain in some countries. The P serotype of all seven serotype G8 strains found in this study was P1B, usually found in serotype G2 human GARV and different from serotype P4 of 69M strain [Matsuno et al., 1985], suggesting that these strains might have evolved by reassortment between a serotype G2 human strain and serotype G8 human or animal strain. Moreover, an analysis of P type of the GARV strains of unusual constellations suggests that these strains may be a reassortant among human rotaviruses, supporting the findings that gene reassortment occurs commonly among human rotaviruses and results in antigenic evolution [Flores et al., 1982; Gerna et al., 1994]. Further genetic analysis such as RNA–RNA hybridization [Ohshima et al., 1990] or RNA sequencing of these strains might be useful to determine the origin of the uncommon G serotypes found in humans.

In Kenya, GARVs were isolated in all the months during the three years in at least one of the three hospitals with obvious peak epidemics occurring during some months. In terms of the peak of the GARV isolation rate, GARVs were detected as frequently as in 58.8% of children with diarrhea in IDH in August 1992, 64% in Nanyuki in October 1991 and 50.0% in Kitui in August 1991. Therefore, GARVs remain one of the important pathogens of acute infantile diarrhea in Kenya which causes patients to visit the outpatient clinics.

GARV infections prevailed during the dry seasons more than the rainy seasons in Nairobi and Nanyuki. However, the seasonality of GARV diarrhea is still not completely clear and climatological changes may not be the only factor that influenced the differences observed [Hieber et al., 1978; Konno et al., 1983; Nakitare et al., 1987]. The GARV detection rate was the lowest in Kitui where little rainfall is observed throughout the year. Another factor may be overcrowding which must play a major role in enhancing GARV transmission confirming the high GARV prevalence found in Nairobi.

Continuous monitoring of the yearly epidemics along with routine surveillance of GARV strains will provide important information on antigenic and genetic changes which may be a prerequisite to the choice of G serotype for GARV vaccines. It also will be important for monitoring vaccine efficacy once the GARV vaccine is incorporated into the regular immunization program commencing soon after birth in Kenya.

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